fragments on a 0.8% agarose gel and blotting onto Hybond-N membrane using standard methods (Sambrook *et al.*, 1989). The M3 cDNA fragment was gel-purified and labelled with [32 P] dCTP as described previously. A Southern blot was hybridized overnight at 65 °C in 6× SSC and washed at 65 °C in three 15 min steps in 0.1% SDS and decreasing salt concentrations (6×, 2× and 0.1× SSC).

Plasmid construction

Recombinant DNA techniques were carried out as described by Sambrook *et al.* (1989) to construct a transcriptional fusion bringing the expression of the *uidA* (commonly named *gus*) reporter gene under the control of the *BnM3.4* promoter in binary vector.

A 'promoterless' gus gene cassette, containing the gus coding sequence (Jefferson et al., 1986) and the nopaline synthase polyadenylation site (nos-ter; Bevan et al., 1983) was cloned into pBluescript SK-(Stratagene, USA), leading to pAF1opt. A 2056 bp BamHI-NspV fragment (Figure 3) from the genomic clone BnM3.4 was cloned into BamHI-ClaI sites of the plasmid pAF1opt, generating pJD51. The chimaeric gene was then excised from pJD51 and introduced into the binary plasmid pEC2 (Cartea et al., 1998) in inverse orientation with respect to the Basta resistance gene, generating pJD101. The promoterless gus gene cassette was also transferred into pEC2 to generate the negative control binary plasmid pAF100. The binary plasmids pAF100 and pJD101 were introduced into Agrobacterium tumefaciens strain C58C1 (Koncz and Schell, 1986) by electroporation (Nagel et al., 1990; Singh et al., 1993). The recombinant genes were introduced into A. thaliana by in planta infiltration (Bechtold et al., 1993).

In situ hybridization

The *BnM3.4* coding sequence was cloned in both orientation in pGEM-3Zf(+) (pJD6 and 7). After *SmaI* linearization, these two plasmids were used to synthesize digoxigenin-11-rUTP-labelled probes using the Riboprobe Combination System T7 kit from Promega.

B. napus floral buds were fixed in 4% formaldehyde, embedded in wax, and 8 μ m-sections were prepared for *in situ* hybridization according to Jackson (1991).

Full-length RNA probes were alkaline-hydrolysed to 150 nt fragments and hybridized to sections at a concentration of 0.5 ng/ml per kb of probe. Hybridization was carried out in 210 μ l for a sandwich of

two slides at 50 °C in 50% formamide, 10% dextran sulfate, 1× Denhardt's solution, 0.3 M NaCl. 10 mM Tris-HCl pH 8, 1 mM EDTA, 1 mg/ml yeast transfer RNA. Slides were washed in several baths of 0.2× SSC at 55 °C for 1 h, followed by two rinses of 5 min each with 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (NTE buffer), and treated with 20 mg/ml RNase A in this buffer at 37 °C for 30 min. The slides were then washed again in NTE buffer and 0.2× SSC as described above, and finally washed in PBS (130 mM NaCl, 7 mM Na₂HPO₄, 3 mM NaH₂PO₄, pH 7) for 5 min. Immunological detection of the hybridized probe was carried out as described in the Boehringer digoxigenin-nucleic acid detection kit with some modifications. Slides were incubated with gentle agitation for 1 h in 0.5% blocking agent (Boehringer) in buffer 1 (100 mM Tris-HCl, 150 mM NaCl, pH 7.5) followed by 1 h in 1% bovine serum albumin, 0.3% Triton X-100 in buffer 1 (buffer 2). This was followed by a 1 h incubation in dilute antibody conjugate (Boehringer) (1:1250) in buffer 2 and 4 washes of 15 min each in buffer 2. Slides were briefly washed in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM MgCl₂ and incubated for 12-48 h in 0.34 mg/ml nitroblue tetrazolium salt and 0.175 mg/ml 5-bromo-4-chloro-3indoyl phosphate toluidinium salt in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM MgCl₂. The colour reaction was stopped with 10 mM Tris-HCl pH 8.0, 1 mM EDTA, and sections were passed through an ethanol series before mounting in mounting medium from Sigma.

Histochemical GUS assays

Histochemical assays for GUS activity were conducted according to the protocol described previously by Jefferson *et al.* (1987) with some modifications. Fresh tissue samples were fixed in a solution containing chloroform, 95% ethanol and water in proportions 3:6:1 and 0.1% Triton X-100 under vacuum (-93.3 kPa) for 1 min. Then, tissue samples were washed twice in 50 mM potassium phosphate buffer, pH 7.0. GUS staining was performed by vacuum infiltration (3× 10 min at -93.3 kPa) in the GUS stain solution (Jefferson *et al.*, 1987) and by incubating at 37 °C overnight. The plant material was then cleared by rinsing with 70% ethanol and the samples were examined and photographed under a Diaplan type 307-148.002 microscope (Leitz, Wetzlar, Germany).

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